LYSOPHOSPHOLIPID INDUCED VOLUME CHANGES IN LYSOSOMES AND IN LYSOSOMAL LIPID DISPERSIONS

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SUMMARY

A Coulter Counter is used to determine volume changes induced by lysophospholipids in lysosomes, in lysosomal lipid dispersions and in dispersions of pure egg yolk lecithin with added lysolecithin. In each case the increased lysophospholipid content causes an increase in the particle volume along with a decrease in the total number of particles. It is suggested that these volume changes may result from lysosomal or lipid membrane fusion induced by the lyso-compounds.

INTRODUCTION

The process of membrane fusion has been studied in a wide range of systems and has been reviewed recently by Poste and Allison (1). The specific importance of fusion in the function of lysosomes has been discussed in detail by deDuve and Wattiaux (2) and by Lucy (3). They suggest that membrane fusion allows primary lysosomes to act upon and digest specific extracellular material both in the cell and in certain circumstances in the extracellular space. Recently Raz and Goldman (4) have observed spontaneous fusion of rat liver lysosomes in-vitro using techniques of electron microscopy. The physico-chemical basis of membrane fusion has been discussed by Dingle (5) in lysosomes and by Lucy and coworkers (6,7) in a variety of systems including hen erythrocytes, mouse fibroblasts and artificial lipid systems. Lucy (3) has proposed the hypothesis of the interdigitation of globular micelles of lipid of two adjacent membranes as one mechanism of fusion. He has provided evidence that the existence of such a micellar organization in place of the bimolecular leaflet structure would be enhanced by the presence of lysophospholipids or other surface active lipids such as unsaturated fatty acids. He further suggests that these compounds may enhance

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fusion by increasing the fluidity of the hydrocarbon chains of the phospholipids thereby altering the orientation of the polar heads. deDuve and Wattiaux (2) point out that such fusion or coalescence might be enhanced if the adjacent membranes are of similar chemical compsition, and it is interesting that the lipid composition of the lysosomal membrane closely resembles that of the plasma membrane (8).

This report presents evidence that lysosomes and lysosomal lipid dispersions increase in volume as a function of the content of surface active lipids (lysophospholipids and free fatty acids) in the membrane. It is suggested that these volume changes may occur as a result of membrane fusion. Weglicki and coworkers (9) have studied the effect of thermal stress at acid pH on the lipid composition of lysosomes. The incubation of the lysosomes at 37°C, pH 5, caused a concomitant increase in lysophospholipids and free fatty acids, and the liberation of β-glucuronidase (9,11). The production of lysophosphatidyl choline (LPC) and lysophosphatidyl ethanolamine (LPE) at acid pH results from activation of acid-active lysosomal phospholipases (10); acid-active triglyceride lipases and phospholipases both contribute to the significant release of free fatty acids (11).

MATERIALS AND METHODS

Rat liver lysosomes containing Triton WR 1339 were isolated (11) and then incubated in a shaking bath at 37°C for varying amounts of time in acid buffer (.25M sucrose, 0.04M Tris acetate, pH 5.0). The lysosomes were used for direct measurements of size and number, or the lipids were extracted (12) for lipid dispersion studies and lipid analysis (11). In the last set of experiments, lecithin was purified from fresh egg yolks by chromatography on silicic acid (13). The lysolecithin was purchased from Sigma Chemicals. All experiments (after initial incubation) were carried out at room temperature in a buffer medium of "Isoton" (Coulter Electronics). Experiments were performed on a Coulter Counter (Model ZBI) along with a companion Channelyzer which provided a readout of size distribution.

RESULTS AND DISCUSSION

The first attempt to size these lysosomes is presented in Table 1 and demonstrates a specific increase in the mode volume of the lysosomes during the course of incubation. As the content of lysophospholipids increase along with an increase in the free fatty acids, the mode volume

TABLE 1

Changes in Lysosome Volume and Lysophospholipid Content After Incubation at 37°C, pH 5.0

Incubation Time	% Lysophospholipid (of total lipid)	Mode Lysosomal Volume $\mu^3 \pm$ S.D.
0	2.1	.14 ± .01
30'	18.7	.24 ± .02

of the lysosomes increases from .14μ^3 to .24μ^3. At these volumes with mean diameters of slightly over .5μ, the Coulter Counter is being used at the lower limit of its capacity to measure size. Because of the noise generated by dust and sample contamination in this range, it is only possible to determine accurately the peak size of the lysosomes. This noise allowed only for a rough estimate of particle number with a standard deviation of about 50%. In each case, however, when the particle volume increased there was a proportional decrease in the total number of particles. It is interesting to note that the lysosome volumes determined using the Coulter Counter are in excellent agreement with the values calculated from the micrographs of Raz and Goldman (4) before and after spontaneous lysosome fusion.

Alternatively we prepared aqueous dispersions of lipid or "liposomes" -according to the method of Bangham (14)- from the lipid extracts of lysosomes that had been previously incubated at 37°C, pH 5.0 for 0, 20 and 30 minutes. Figure 1 and Table 2 indicate that as the content of lysophospholipids increases in the lysosomal lipid extracts, the derived liposomes are larger and the total number of particles is smaller. This data clearly suggests that the increased content of lysophospholipids is causing the formation of larger and fewer liposomes. The possibility that this change represents only swelling of some of the particles and lysis of others is unlikely because of the correlation between particle size and number. Furthermore, in each case the curves are monophasic with no increase in particle debris as the lysophospholipid levels increase.

We approached the question of whether the change in lysophospholipid content of the lysosomal preparation is the sole cause of these striking

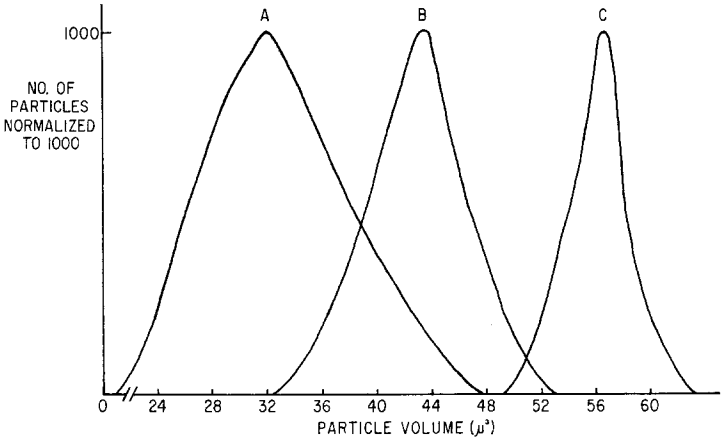

FIGURE 1. Volume of Liposomes derived from dispersions of lysosomal lipids. Before lipid extraction, lysosomes were incubated at 37°C, pH 5.0 for 0 min. (A), 20 min. (B) and 30 min. (C). Each sample has varying amounts of lysophospholipids as shown in Table 2. Number of particles are normalized to 1000 by the Coulter Channelyzer.

TABLE 2

Volume of Liposomes Derived from Lysosomal Lipid Extracts

Incubation Time Min.	% Lysophospholipids (of total lipids)	Mode Volume μ^3 ± S.D.	No. of Particles Integrated Under Curve ± S.D.
0	2.1	32.0 ± 2.1	15,680 ± 280
20	15.1	43.2 ± 1.2	11,486 ± 165
30	18.7	56.6 ± 1.1	6,862 ± 120

Volume and number of liposomes derived from lysosomal lipid extracts. Before lipid extraction, lysosomes were previously incubated for varying times at 37°C, pH 5.0.

changes in particle size and number, by looking at the effect of pure lysolecithin on an aqueous dispersion of pure egg lecithin (liposomes). 400 mgm of the pure lipid was added to 15 ml of the diluting buffer ("Isoton") in each of two round bottom flasks. Both flasks were hand shaken for 15 minutes or until all of the lipid was dispersed and then placed on a slow speed shaker at room temperature (21°C) under nitrogen.

50 mgm of lysolecithin was then added to one of the flasks, and at varying time periods 50 1 of each were sampled and sized on the Coulter Counter. The results are presented in Table 3. Initially there is no

TABLE 3

Volume Changes in Egg Lecithin Dispersions With Added Lysolecithin

Time (hrs)	Volume in μ^3	
	Lecithin Dispersion	Lecithin Dispersion + Lysolecithin
0	75.7	75.5
4	75.2	79.2
12	75.7	94.0
24	77.7	105.3
48	74.9	111.3

Changes in volume of lecithin liposomes with time after addition of lysolecithin in buffer. (1 experiment typical of 3)

difference between the two samples with respect to size. After 48 hours there has been about a 50% increase in the average particle or liposome size. This increase in particle volume is accompanied by a 35% decrease in the total particle number for the lysolecithin treated sample with no significant change in the control. The time course of particle growth is consistent with the idea that the lysolecithin when added to the aqueous phase, forms small globular micelles that may exchange with the liposomes at a relatively slow rate.

This study has indicated that in three separate systems lysophospholipids may produce a significant increase in particle size along with a decrease in the total number of particles. From this point of view it is not possible to distinguish between fusion as a result of surface coalescence or an actual mixing of the intracellular contents which is a more useful definition of fusion. The work of Ahkong and coworkers (15) and of Klibansky and deVries (16) indicates that in avian erythrocyte systems, the microscopically observed fusion involves the actual mixing of the

cytoplasm as large multinucleated cells form. In both of these cases the fusion is induced by added lysolecithin.

Recent preliminary experiments with single bilayer lecithin vesicles indicate that lysolecithin induced fusion actually involves the mixing of the contents of specific vesicles. These experiments were performed by looking at a colorimetric reaction that occurs only between the contents of two specific groups of vesicles after fusion and mixing of the contents has occurred. These results are in good agreement with the work of Taupin and McConnell (17) using electron spin resonance techniques on similar model membrane systems.

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